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Significance of the intraspecific morphological variability in biomonitoring studies with mosses: Among-populations and between-sexes approach

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ABSTRACT

In this study we assessed for the first time the intraspecific morphological variation in gametophytes of the terrestrial moss *Pseudoscleropodium purum* in populations growing naturally in areas affected by different levels of atmospheric pollution (2 industrial and 2 unpolluted sites). We also identified the sexes in each population and evaluated the morphological variation in male, female and non-expressing plants, together with the between-population variation in levels of sex expression and the sex ratio (female: male). Although sex expression levels and sex ratios varied between populations, neither of these variables were significantly correlated with the levels of pollution at any of the four sites. We therefore conclude that the reproductive traits of this species are not affected by the pollution levels considered in the study. We observed significant between-population variation in 8 of the 20 morphological traits considered, although the variation was not associated with male, female or non-expressing plants. The morphological traits were not significantly correlated with the levels of heavy metals in the study sites. However, the plants from the most polluted sites (the industrial environments) were generally smaller (shorter plants with fewer branches and smaller leaves) and therefore had less surface area available for uptake of atmospheric pollutants than the plants from the other sites. Irrespective of the factors underlying the variation, mosses from some populations can accumulate more pollutants than mosses from other populations (consistent with their morphological characteristics). This source of variation in the concentrations of heavy metals measured in moss biomonitoring studies has not previously been taken into consideration.

1. Introduction

Monitoring the spatial and temporal trends in airborne heavy metals with native terrestrial mosses has been undertaken for many years with the aim of assessing the extent and intensity of pollution in different areas and at diverse spatial scales (e.g. Uyar et al., 2008; Harmens and Norris, 2008; Boquete et al., 2009). Some studies have been carried out in parallel to the moss biomonitoring technique, to identify the factors that enhance the uncertainty of the results obtained, e.g. environmental conditions (such as rain events), topographical features (such as elevation), presence of vegetation and point sources of pollution, and different sample processing procedures (for review see Fernández et al., 2015). Several of these studies reported that the species of moss used was one of the most important factors determining the final concentrations of pollutants obtained in biomonitoring studies with terrestrial mosses (Kleppin et al., 2008; Schröder et al., 2008; Holly et al., 2009). It has been recently demonstrated that four of the seven most important factors affecting the overall uncertainty of the results of biomonitoring studies are related to the moss species or moss material used (Dolegowska and Migaszewski, 2015): (i) the amount of moss collected; (ii) the growth rates of moss tissues; iii) the part of moss analyzed; and (iv) interspecific variations linked to moss morphology.

Terrestrial mosses capture elements from the environment over the entire surface of the gametophyte (Brown, 1982), which is the dominant generation in the moss life cycle. The morphological characteristics of the gametophyte therefore largely determine the capacity of mosses to capture elements. Morphological features determine the surface area to volume ratio and therefore the

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surface area per unit volume in contact with the environment and thus available to capture and retain both nutrients and atmospheric pollutants. Differences in the structure of the gametophyte may thus cause differences in the uptake and retention capacities of mosses (Vanderpoorten and Goffinet, 2009). Such differences in the capacity of mosses to accumulate atmospheric pollutants have been shown to exist between species with different growth and life forms (e.g. Fabure et al., 2010) as well as between species with similar growth and life forms (e.g. Halleraker et al., 1998; Galsomiès et al., 2003; Castello, 2007; Carballeira et al., 2008). Regarding different growth and life forms, acrocarpous mosses form small tufts and cushions, are erect, and generally unbranched or slightly branched, while pleurocarpous mosses are often prostrate, highly branched and form quite broad carpets (Glime, 2013), which implies higher soil coverage and makes these mosses preferable to acrocarpous species for biomonitoring studies.

The results of studies assessing the accumulation capacities of different moss species with similar growth and life forms are variable. In a study comparing the concentrations of several heavy metals in transplants of *Hypnum cupressiforme* Hedw. and *Pseudoscleropodium purum* (Hedw.) M. Fleish. exposed at several sampling points to different levels of pollution, Castello (2007) found that the concentrations were generally higher in *P. purum*. This author suggested *P. purum* might be able to capture particles more efficiently because of its broader and less crowded leaves, while *H. cupressiforme*, with smaller and more closely packed leaves would preferably accumulate wet deposited pollutants owing to its higher water retention capacity. Conversely, Carballeira et al. (2008) suggested that native *H. cupressiforme* accumulates heavy metals more efficiently than *P. purum* because of the larger specific surface area of the former (related to the smaller diameter of the stem of *H. cupressiforme*), among other reasons. In an earlier study, Halleraker et al. (1998) pointed out that the smaller leaves and greater number of branches in *H. cupressiforme* than in *Pleurozium schreberi* (Brid.) Mitt. would enable the former to capture minerogenic elements more efficiently. Despite the variability in results, the evidence indicates that morphological differences between species significantly affect the concentrations of atmospheric pollutants determined in biomonitoring studies.

The morphological variation in bryophytes is not restricted to the between-species level, but also occurs at the within-species level. This intraspecific variation has been observed along latitudinal gradients (e.g. for *Hylocomium splendens* as shown by Montagnes and Vit, 1991), and also at smaller scales along humidity (Wigh, 1975, 1976), temperature and water chemistry gradients. The high plasticity of some morphological characters in moss gametophytes constitutes an important problem for establishing species boundaries in bryophyte taxonomy (when restricting species identification to a number of gametophyte characters) (Kungu et al., 2007). Hence, intraspecific variation in morphology is also expected to influence the results of biomonitoring studies of air quality with terrestrial mosses. However, we are unaware of any biomonitoring study in which this variable has been assessed at the intraspecific level.

Pseudoscleropodium purum is a pleurocarpous moss belonging to the Brachitheciaceae family and is commonly used in biomonitoring studies (e.g. Krommer et al., 2007; Suchara et al., 2011; Boquete et al., 2015). The species is dioecious, which means that the gamete-producing organs (male and female gametangia) occur in separate plants. In light of this and the above information, the main aim of this study was to establish any differences in some selected morphological characters of P. purum growing naturally in areas affected by different levels of atmospheric heavy metal deposition. These differences were assessed at the between population level (comparing morphological traits of P. purum gametophytes in four populations exposed to increasing levels of heavy metals deposition), as well as between sexes within the same population (comparing morphological traits of males, females and non-expressed plants within each population). Besides, we evaluated whether heavy metal inputs were related to the levels of sex expression and sex ratios in this species and discussed the impact of any such differences on biomonitoring studies with terrestrial mosses

2. Material and methods

In January 2014, samples of the terrestrial moss *P. purum* were collected in four sites in Galicia (NW Spain) affected by different degrees of pollution. Thus, two native populations were sampled in the surroundings of metallurgical plants (an Fe-smelter, Site 1, and an Al-smelter, Site 2), which are well known sources of heavy metals to the atmosphere. Another two native populations were sampled in unpolluted areas (Site 3 and Site 4). The heavy metal levels in *P. purum* from these four sites have been monitored over several years (1999–2012) and the data reported in various articles (Varela et al., 2014; Boquete et al., 2011, 2013, 2015) (for a summary see Table 1). Entire shoots of *P. purum* were sampled evenly, over an area of $1 \times 2\ m^2$ at each site, and transported to the laboratory in plastic bags. Whole moss shoots were separated from foreign material, air-dried at 15 °C for 3–5 days, and stored in paper bags until further processing.

Table 1
Median and median absolute deviation (MAD) of the concentrations of various atmospheric pollutants ($\mu g\ g^{-1}$; *: $ng\ g^{-1}$) in samples of *Pseudoscleropodium purum* collected during the study period in sites 1–4. Period: time during which *P. purum* was collected in each of the sites; n: number of data available for each element and sampling site. The data for the different sites were obtained from various articles: S1 (Varela et al., 2014; Boquete et al., 2013; Boquete et al., 2015); S2 (Varela et al., 2014; Boquete et al., 2011; Boquete et al., 2013; Boquete et al., 2015); S3 (Boquete et al., 2011; Boquete et al., 2013; Boquete et al., 2015).

Site	Period		Al	As*	Ba	Be*	Cd*	Co*	Cr	Cu	Fe	Hg*	Mn	Ni	Pb*	Se*	Sn	Sr	V*	Zn
S1	June-04–January-12	n	6	7	6	6	27	8	8	27	8	27	6	8	27	7	1	6	8	27
		Median	973	803	35.9	80.4	1512	1529	24.7	33.0	4410	612	392	25.5	92944	59.0	615	30.5	3390	351
		MAD	150	139	3.78	65.1	398	290	2.82	5.94	950	150	53.4	11.4	32277	13.6		25.9	507	65.3
S2	November-99–December-10	n	105	4	4	4	124	4	105	124	105	122	105	105	124	4	–	4	105	124
		Median	2224	250	23.1	24.5	290	352	20.9	8.15	652	71.1	38.9	15.3	3556	176	–	33.9	9111	55.5
		MAD	597	74.4	1.28	10.8	39.4	98.9	9.14	0.79	194	8.60	8.99	3.52	615	19.9	–	142	1110	6.45
S3	May-05–December-05	n	62	–	–	–	62	–	62	62	62	62	62	62	62	–	–	–	62	62
		Median	410	–	–	–	51.5	–	12.4	6.19	293	38.8	219	16.1	2033	–	–	–	2475	32.3
		MAD	126	–	–	–	6.02	–	4.03	0.60	89.2	4.54	23.5	3.71	357	–	–	–	412	5.2
S4	February-08–December-10	n	–	–	–	–	19	–	–	19	–	19	–	–	19	–	–	–	–	19
		Median	–	–	–	–	66.4	–	–	5.01	–	29.7	–	–	1549	–	–	–	–	45.5
		MAD	–	–	–	–	4.70	–	–	1.08	–	2.45	–	–	531	–	–	–	–	9.77

These data were obtained from: S1: Varela et al., 2014; Boquete et al., 2013; Boquete et al., 2015. S2: Varela et al., 2014; Boquete et al., 2011; Boquete et al., 2013 and Boquete et al., 2015; S3: Boquete et al., 2011; Boquete et al., 2013 and Boquete et al., 2015.